ROUGHOID, A MUTANT LOCATED TO THE LEFT OF SEPIA IN THE THIRD CHROMOSOME OF DROSOPHILA MELANOGASTER.

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ORIGIN OF THE CHARACTER, ROUGHOID (ru).

In March, 1919, Dr. A. H. Sturtevant handed over to me a culture of *Drosophila melanogaster*, containing a new mutant, named by him "roughoid," owing to its similarity with the old character "rough."

In December, 1918, he had collected a single pair of wild flies near Columbia, S. C. The F_2 generation from these was found, two months later (February 14, 1919), to contain many roughoids (culture 5570 A.H.S.). Hence one of the wild flies must have been heterozygous for the mutant gene. The point of special interest in connection with roughoid is that its locus is to the left of that of sepia and therefore establishes a new left end of the map of the third chromosome.

DESCRIPTION OF THE ROUGHOID CHARACTER.

This gene produces somatic effects mainly upon the size and texture of the normal eye; (*a*) it materially decreases both the length and width of the eye as well as making it more convex; (*b*) the ommatidia lose their hexagonal shape, and become crowded together irregularly; (*c*) due to this irregularity, the hairs project in all directions from the surface; (*d*) the hairs are somewhat thickened and strikingly longer than in the normal eye. These effects are very constant in appearance and have proven to be favorable for purposes of classification. Associated with these effects there are found sometimes, (*e*) several black ommatidia distributed over the surface of the eye, but more commonly in the posterior ventral region. They tend to darken the normal red eye color. They are more than likely a part of the roughoid character since I have never seen this effect in any

fly other than one that was also roughoid. However, their occurrence is very inconstant, most roughoid eyes being as light red as the normal wild type.

Chromosome Carrying Roughoid.

The first experiment (culture No. 142) consisted in out-crossing a roughoid male to a female showing the second-chromosome recessive vestigial.[1] The F_1 generation flies were all wild type, thus proving roughoid to be a recessive character. By inbreeding the F_1 flies, F_2 was obtained. One quarter of the F_2 vestigials showed the roughoid character (culture No. 142*b*). The presence in F_2 of the double recessive class proves that roughoid cannot be located in the second chromosome. At the same time, I out-crossed a roughoid female to a dichæte hairless male from stock (culture No. 141). These two characters are third-chromosome dominants. By back-crossing the F_1 dichæte hairless females to roughoid males from stock, evidence was obtained that roughoid shows linkage relations with these mutants in the third chromosome. The size relation of the various classes indicated that roughoid was considerably to the left of dichæte. As the back-cross flies were not hatching out as well as expected, I did not complete the count of the cultures of this experiment.

A cross between roughoid and the original rough gave in F_1 only wild-type flies, showing that roughoid was neither rough nor an allelomorph of rough.

Location of Roughoid in the Third Chromosome.

The next step was to make up a stock containing both the new character, roughoid, and sepia (located at the extreme left end of the then known third chromosome). In the third generation from a cross between roughoid and sepia, I obtained such a stock. Since I had obtained roughoid and sepia together in such a short time, I concluded that the two were not very closely linked. By using the back-cross method, I obtained the following results:

[1] This female was from the stock known as "5 ple" and carried in addition to vestigial, the second-chromosome recessives black, purple, arc and speck. These other characters were disregarded in the F_2 classifications.

$$\frac{ru\quad se}{\varphi} \times \sigma^{\prime} \frac{ru\quad se}{+\quad +} \qquad \frac{ru\quad se}{ru\quad se}.$$

July 24, 1919.

Culture No.	ru se.	+	ru.	se.	Total.	Crossovers.	Crossover Value.
601	133	79	31	27	270	58	21.5
602	99	98	38	37	272	75	27.5
606	106	87	33	37	263	70	26.6
607	56	68	26	15	165	41	24.8
608	71	67	22	26	186	48	25.8
Total	465	399	150	142	1156	292	25.2

$$\frac{ru\quad +}{\varphi} \times \sigma^{\prime} \frac{ru\quad se}{+\quad se} \qquad \frac{ru\quad se}{ru\quad se}.$$

May 24, 1919.

Culture No.	ru.	se.	ru se.	+	Total.	Crossovers.	Crossover Value.
405	71	77	16	36	200	52	26.0

The total of these cultures (1,356 flies) shows the cross-over value for roughoid and sepia to be 25.3.

Again, a dichæte female (known to be heterozygous for roughoid) was backcrossed to a roughoid male from stock. From that experiment, I found the cross-over value for roughoid and dichæte to be 35.9, as follows:

$$\frac{ru\quad D}{\varphi} \times \sigma^{\prime} \frac{ru\quad +}{+\quad +} \qquad \frac{ru\quad +}{ru\quad +}.$$

July 10, 1919.

Culture No.	ru D.	+	ru	D.	Total.	Cross-overs.	Cross-over Value.
605	59	64	41	28	192	69	35.9

This indicates that roughoid is situated to the left of sepia, since the normal distance between sepia and dichæte is known to be about 11.7 units.

Three-Point Back-Cross—ru se × D.

In order to place the gene more accurately, a three-point back-cross was undertaken. A sepia roughoid female was crossed to a dichæte male, the F_1 dichæte females were then back-crossed to roughoid sepia males from stock (pair matings being made). Results:

$$\text{♀}\ \frac{ru\ \ se\ \ +}{+\ \ +\ \ D} \times \text{♂}\ \frac{ru\ \ se\ \ +}{ru\ \ se\ \ +}\ .$$

July 7, 1919.

Culture No.	0.		1.		2.		1, 2.		Total.
	ru se.	D.	ru D.	se.	ru se D.	+	ru.	se D.	
604	82	82	17	30	16	18	1	0	246
611	45	51	15	24	14	12	2	0	163
Total	127	133	32	54	30	30	3	0	409
	260		86		60		3		

The results give a cross-over value for roughoid and sepia of 19.5 and 25.1 respectively, or a mean of 21.7; a cross-over value for sepia and dichæte of 14.2 and 17.2 or a mean of 15.4.

Four-Point Back-Cross—ru se × D H.

One other back-cross experiment was performed, using the four characters roughoid, sepia, dichæte and hairless. From this, 980 flies were counted, giving the cross-over value for roughoid and sepia as 25.6.

$$\text{♀}\ \frac{ru\ \ se\ \ +\ \ +}{+\ \ +\ \ D\ \ H} \times \text{♂}\ \frac{ru\ \ se\ \ +\ \ +}{ru\ \ se\ \ +\ \ +}\ .$$

August 12, 1919.

Culture No.	0.		1.		2.		3.		1, 2.		1, 3.		2, 3.		1, 2, 3.	
	ru se.	D H.	ru D H.	se.	ru se D H.	+.	ru se H.	D.	ru.	se D H.	ru D.	se H.	ru se D.	H.	ru H.	se D.
609	52	64	23	24	17	14	11	14	2	0	10	6	1	6	2	1
610	53	64	21	18	16	9	22	18	0	0	3	5	4	6	0	2
612	30	54	14	19	10	16	7	18	1	0	6	7	4	3	0	0
Total	135	182	58	61	43	39	40	50	3	0	19	18	9	15	2	3
	317		119		82		90		3		37		24		5	

$$\text{♀}\ \frac{ru\ +\ D\ H}{+\ se\ +\ +} \times \text{♂}\ \frac{ru\ se\ +\ +}{ru\ se\ +\ +}.$$

May 24, 1919.

Culture No.	0.		1.		2.		3.		1, 2.		1, 3.		2, 3.		1, 2, 3.	
	ru D H.	se.	ru se.	D H.	ru.	se D H.	ru D.	se H.	ru se D H.	+ .	ru se H.	D.	ru H.	se D.	ru se D.	H.
404.....	61	75	24	42	25	20	20	9	0	0	4	12	1	4	1	5
	136		66		45		29		0		16		5		6	

Summing up the data in the previous tables:

Culture No.	Total Flies.	ru se Value.	se D Value.	D H Value.
404.....	303	29.0	18.4	18.4
609.....	247	28.3	17.4	20.6
610.....	241	20.3	15.3	24.9
612.....	189	24.8	17.9	23.8
Total.....	980	25.6	17.3	21.6

The value for dichæte hairless (21.6) is approximately the expected result (24.8). Sepia dichæte crossing over is noticeably high, since the average normal value is 11.7 units. This is possibly due to a complicating factor, linked to the new factor, roughoid, and "stretching out" that part of the third chromosome. More work will have to be done before this can be certain. It will be noticed that the double cross-over class (1, 2) is lower than the triple cross-over class (1, 2, 3). This is unexpected and remains unexplained at the present time.

Summary.

(*a*) A new mutation has occurred in the third chromosome of *Drosophila melanogaster* to the left of sepia, which for several years has been the leftmost of the known loci.

(*b*) There was a roughoid sepia cross-over value of 24.9, based on a total of 2,748 flies of which 685 were cross-overs.

(*c*) Associated with roughoid, there is possibly a linkage modifier increasing the crossing over between sepia and dichæte, and perhaps between roughoid and sepia as well.